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γ-Alkyl- 또는 γ-Benzyl-L-Glutamate가 그라프트된 폴리우레탄의 합성과 혈액적합성 평가

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Synthesis and Blood Compatibility of Polyurethane Grafted with γ-Alkyl- or γ-Benzyl-L-Glutamate

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요 약 : 폴리우레탄에 γ-벤질과 -알킬-L-glutamate가 그라프트된 새로운 공중합체를 합성하고 그 특성을 평가하였다. 펩티드는, PGB의 경우 α-helix구조를, PGE의 경우 β-sheet 구조를 갖으며, PGM의 경우 α-helix와 random coil구조가 혼재하는 것을 FT-IR로 확인할 수 있었다. 그라프트 공중합체의 기계적성질은 펩티드양이 증가함에 따라 감소하였고 함수율은 3% 이하였으며, 혈소판 측정기에 의해 PGB series의 혈소판 접착능이 10% 이하인 것을 확인할 수 있었다. 그라프트 공중합체의 혈액응고 시간은 전반적으로 폴리우레탄과 펩티드 보다 길었다. α-helix구조를 갖는 PGB series의 혈액응고시간은 80분 이상을 나타내었다.

Abstract : Novel graft copolymers consisting of commercial polyurethane (Pellethane 2363 80 AE) grafted with the γ-benzyl and -alkyl-L-glutamate were synthesized and their mechanical properties were studied. The peptide units formed an α-helical conformation, a β-sheet conformation and a combination of α-helical and random coil conformation for polyurethane grafted with γ-benzyl-L-glutamate (PGB), polyurethane grafted with γ-ethyl-L-glutamate(PGE) and polyurethane grafted with γ-methyl-L-glutamate(PGM), respectively, as evidenced by FT-IR analysis. The tensile strength and elongation of the graft copolymers decreased with increasing peptide content. The degree of water absorption of the samples was less than three percent. The platelets adhesion performance for the PGB series was less than ten percent as investigated by the Coulter counter. From the SEM pictures, fibrin network and adhered platelets on the surface of PGB series were not seen. The clotting time of the graft

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+We dedicate this work to the late Professor Kea Yong Kim with very greatful mouring of his sudden passaway.

copolymers was longer than that of polyurethane or homopolypeptide. The PGB series having an α-helical conformation exhibited the clotting time of over eighty minutes.

INTRODUCTION

Polyurethane(PU, Pellethane 2363 80 AE, medical grade) is an unique class of polymers because of its good physical and chemical properties. Polyurethane is also widely used in blood contacting applications as prostheses,[1] implants,[2] devices[3] and organs.[4]

Synthetic peptides have been known to be promising as biomedical materials, because the modified protein components of natural tissue or organs have been utilized in the reconstructive surgery and also known to possess a biocompatibility.

The preparation of graft and block copolymers containing peptide units has been attempted by us to obtain biomaterials with improved antithrombogenic properties.[5~8] We have been investigated on the blood compatibility of the block and graft copolymers consisting of polyurethanes, polypropylene oxide, polyethylene oxide, polyethylene and silicone as the main trunk polymers, and several amino acids as the other components. The results of previous studies showed that the mechanical properties and blood compatibility of the polymeric materials was affected by the peptide units in the copolymers. The blood compatibility of graft copolymer was greatly influenced by the side chain mobility and the conformation of the grafted peptides.

The main objectives of the present study are to synthesize the graft copolymers consisting of polyurethane and peptide units and to investigate the mechanical properties and the blood compatibility of the peptide grafted polyurethanes.

In this study, γ-benzyl or -alkyl-L-glutamate N-carboxyanhydride(NCA) is prepared. Peptide grafted polyurethane is also synthesized by reaction of polyurethane with NCA. The mechanical properties and blood compatibility of the copolymers obtained are investigated in relation to the conformation of the peptide segments.

EXPERIMENTAL

Materials

Polyurethane(PU, Pellethane 2363 80 AE, medical grade) was obtained from Dow Chemical Co. L-glutamic acid and γ-ethyl-L-glutamate(Sigma Chemical Co)., trichloromethyl cholrofomate(TCF, Hodogawa Co.) and sodium hydride(ab. 60% in oil)(Wako Pure Chemical Industries) were used without purification. Methanol, benzyl alcohol, hydrochloric acid and sulfonic acid were also used as received. Dimethyl formamide, tetrahydrofuran, ethylacetate, n-hexane, dioxane and benzene were dried and purified with a usual solvent purification process.

Esterification and Phosgenation of L-Glutamic Acid

The esterification of the γ-carboxyl group of the L-glutamic acid was carried out by the usual method.[7,9] The γ-benzyl and γ-alkyl-L-glutamate were phosgenated in tetrahydrofuran at 45℃, giving rise to crude NCAs(compound **1** in Fig. 1). The crude NCAs(**1**) were purified by recrystallization from the solution of ethylacetate and n-hexane.[10]

Synthesis of Graft Copolymers

A schematic diagram of the synthetic route of graft copolymers was given in Figure 1. The N-substitution was carried out according to the procedure described by Blumstein.[11] A three-necked, round-bottom flask was equipped with a stirrer and a nitrogen inlet. The reaction flask was placed in an ice bath. An appropriate amount of sodium hydride, as a 60% suspension in mineral oil, was added to the reaction flask with pre-cooled polyurethane(**2**) solution(in dimethyl formamide) kept below 0℃ for one hour. Initially, hydrogen was evolved, and a greenish yellow color appeared very rapidly. The color disappeared after the ex-

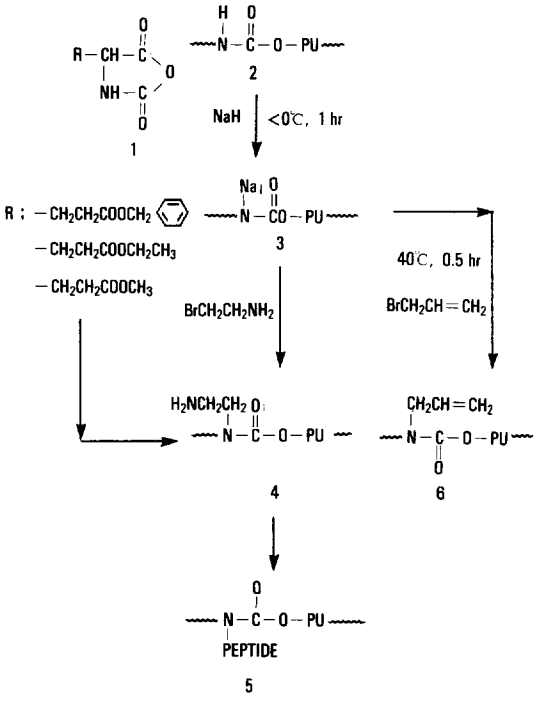

Fig. 1. A schematic diagram of the synthetic route of graft copolymer consisting of γ-benzyl- and -alkyl-L-glutamate and polyurethane.

cess of 2-bromo ethylamine was added to the polyurethane sodium salt(**3**) at 40℃ for half an hour. The solution was precipitated into a large excess of water to remove unreacted sodium halide. The precipitates(**4**) was washed repeatedly with water and methanol. The resulting polymer(**5**) was synthesized by the reaction of PU-s-NH_2(**4**) and γ-protected L-glutamate N-carboxyanhydride(**1**). In this reaction, the substituted amino groups of polyurethane acted as an initiator for the polymerization of NCA.[8] The graft copolymers were washed with benzene and dioxane for several times to remove any homopolypeptides. The results of the graft copolymerization were shown in Table 1. Polyurethane sodium salt(**3**) was reacted with ally bromide for detecting the changed reaction site(**6**), which was controlled to be 0.1mol and 0.01mol calculated from the amount of added allyl bromide.

Table 1. The Graft % and the Mechanical Properties of the Graft Copolymer

Samples	Tensile Strength (kg/mm^2)	Final Elongation (%)	Graft %[a]
PU	2.55	1,104	0
PGB[b]-1	2.10	775	33
PGB-2	1.14	538	55
PGB-3	0.59	330	60
PGB-4	0.45	103	73
PGB-5	0.10	1.1	80
PGM[c]-1	2.36	358	4
PGM-2	2.62	259	32
PGM-3	1.86	196	43
PGE[d]-1	1.41	690	17
PGE-2	1.07	690	29
PGE-3	0.75	200	46

[a] {[wt of copolymer-wt of trunk polymer]/wt of trunk polymer} ×100

[b] Polyurethane grafted with BLG

[c] Polyurethane grafted with MLG

[d] Polyurethane grafted with ELG

Characterization

The conformation of peptide in the graft copolymers was measured by FT-IR(NICOLET, Model 5DX). Scanning electron microscope(SEM, JEOL, Model JSM 35CF) was used to investigate the morphology of the platelets adhered on the polymer surfaces. Coulter counter (Model S-plus) was used to count the number of platelets. The goniometer(Model G-1, Erma Optical Co.) was used to measure the contact angle of polymer samples for calculating critical surface tension.

Degree of Water Absorption

The weight of five dried samples was measured with Sartorius balance Model A200S. These samples were dipped into the test tube filled with deionized water maintained at 37℃ in an incubator for ten days. The degree of water absorption, Q_w (%), of these samples was calculated with the following equation, $Q_w(\%)=[(X_2-X_1)/X_1]\times 100$, where X_1 and X_2 are the weight of dry and swollen samples, respectively.

Critical Surface Tension

The measurement of contact angle was carried out using contact angle goniometer. A drop of organic liquid such as glycerin, formamide, diethylene glycol, oleic aicd, 1,2-dichloroethane and n-butyl alcohol was placed onto polymer films, and the contact angle was measured at least ten different sites of a sample surface. The values of cos θ of the samples were plotted against the surface tension of each liquids used in this test by Zisman method.[12]

Tensile Strength and Elongation

The tensile strength and elongation under dry state were measured according to the method of ASTM D-638 by using Universal testing machine (Toyo Boldwin UTM-4100) at a crosshead speed of 10 mm/min.

Lee-White Method[13]

Polymer samples were coated onto the inside wall of a test tube(15mm×125mm, Corning Ltd.). A 2ml of fresh human blood collected from a healthy male donor of 24 years old was brought into a test tube to contact the coated polymer. The clotting time of five specimens was measured at 37℃ and the test was terminated soon after the blood started to clot.

Column Method[14]

The column method was adopted to count the number of platelets and investigate the morphology of platelet adhered on the polymer surfaces. Glass beads(40-60 mesh) precoated with 1 (w/v) % polymer solution are packed into a glass tube (10 cm in length, 3 mm in inner diameter). Fresh human blood was passed through the tube packed with precoated glass beads for 3 min at a flow rate of 1 ml/min. Passed blood was mixed with anticoagulant(ethylenediamine tetraacetic acid[EDTA]) in the sampling bottle to determine the number of platelets using the Coulter counter(Model S-plus). Glass beads precoated with polymer were in good contact with blood for 20 minutes, and the morphology of platelets adhered on the polymer surfaces is investigated using SEM after the platelets on the polymer surface were fixed with 1.25% glutaraldehyde solution. Platelets adhesion performance was estimated with the average value of five tests of a sample.

RESULTS AND DISCUSSION

Conformation

The detection of amino group in amine substituted polyurethane(**4**) was very difficult due to that the absorption peak of NH and NH_2 overlapped around 3,200cm^{-1} in FT-IR spectrum. Therefore, in this study, to examine the state of reaction of NH to NH_2, allylbromide was used as a model compound(**6**) in the reaction of sodium substituted polyurethane with 2-bromoethyl amine. Figure 2 shows infrared spectra of allyl substituted polyurethanes. The peak intensity of NH stretching vibration appeared at 3,200cm^{-1} was decreased with increasing allyl contents. These spectra indicated that the allyl(or ethylamino) substitution

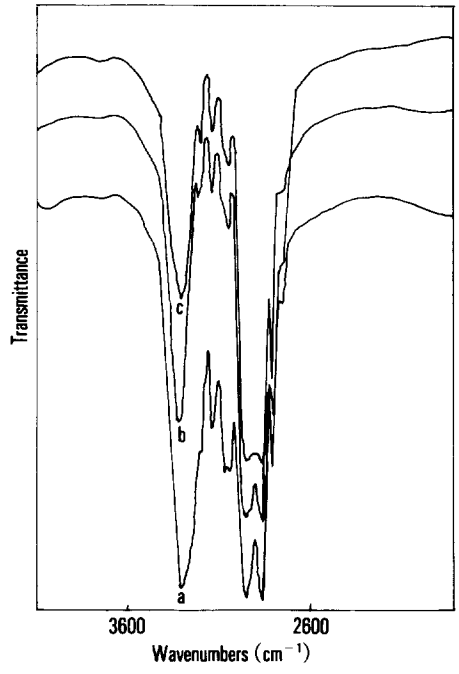

Fig. 2. IR spectra of polyurethane and allyl substituted polyurethane ; a) polyurethane, b) 0.01 mol allyl substituted PU, and c) 0.1 mol allyl substituted PU.

was successfully proceeded.

The conformation of the graft copolymer(5) was identified by FT-IR analysis. Figures 3-6 showed infrared spectra of the graft copolymers. It has already been reported that the characteristic peaks for an α-helical conformation of peptide are shown at 1,650 cm^{-1} and 1,550 cm^{-1} for amide I and amide II, respectively.[15,16] Also the characteristic peaks for a β-sheet conformation appeared at 1,630 cm^{-1} and 1,530 cm^{-1} for amide I and amide II, respectively. And then the characteristic peaks for random coil conformation appeared at 1,650 cm^{-1} and 1,530 cm^{-1} for amide I and amide II, respectively.

Figure 3 showed IR spectra for PGB series having 1,650 cm^{-1}(amide I) and 1,545 cm^{-1}(amide II), where the peptide unit in PGB series had an α-helical conformation. Figure 4 showed the IR spectra of PGB-3 and PGB-5. As shown in Fig. 4, the intensity of N-H peak increased with the content of peptide in the copolymers. On the other hand, PGM series having 1,653 cm^{-1}(amide I) and 1,545 cm^{-1} and 1,533 cm^{-1}(amide II) have a combination of an α-helical and β-sheet conformation as shown in Figure 5, while PGE series having 1,630 cm^{-1} (amide I) and 1,530 cm^{-1}(amide II) show a β-sheet conformation(Figure 6). A difference in conformation may affect the blood compatibility of the respective graft copolymers. In particular, PGB series are expected to exhibit a good blood compatibility because the conformation of peptide segment of the PGB series(helical conformation) is similar to the conformation of peptide in the living body.

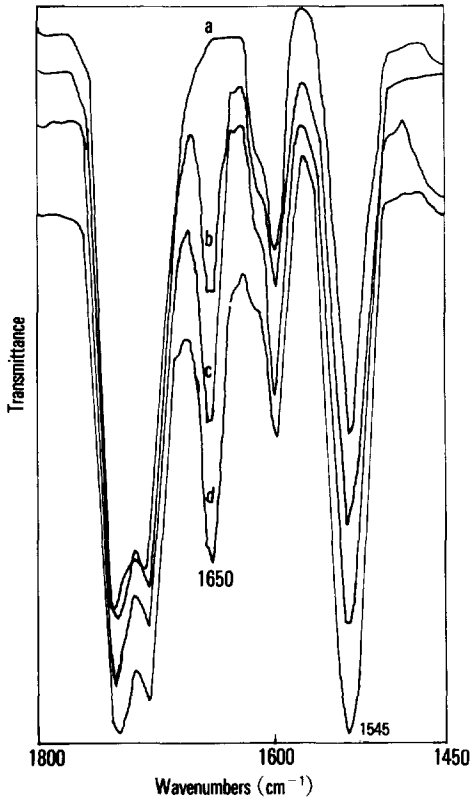

Fig. 3. IR spectra of PGB series ; a) polyurethane, b) PGB-1, c) PGB-3, and d) PGB-5.

Physical Properties

Static mechanical properties of the graft copolymers and polyurethane were measured by Universal testing machine and reported in Table 1. The elongation and tensile strength of the graft copolymers decreased with increasing content of peptide segments of the copolymers. This observation is understandable because the mechanical properties

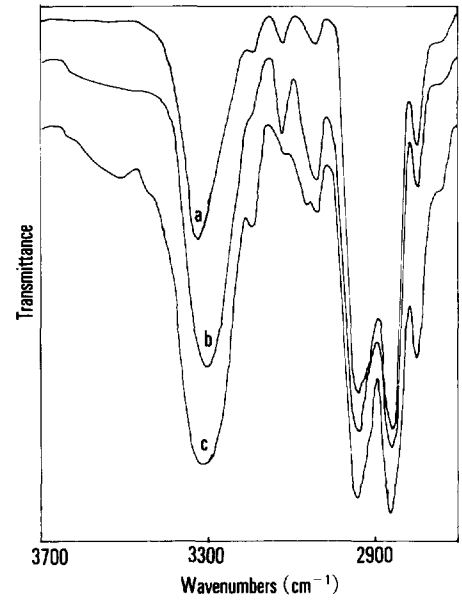

Fig. 4. IR spectra of PGB series ; a) polyurethane, b) PGB-3, and c) PGB-5.

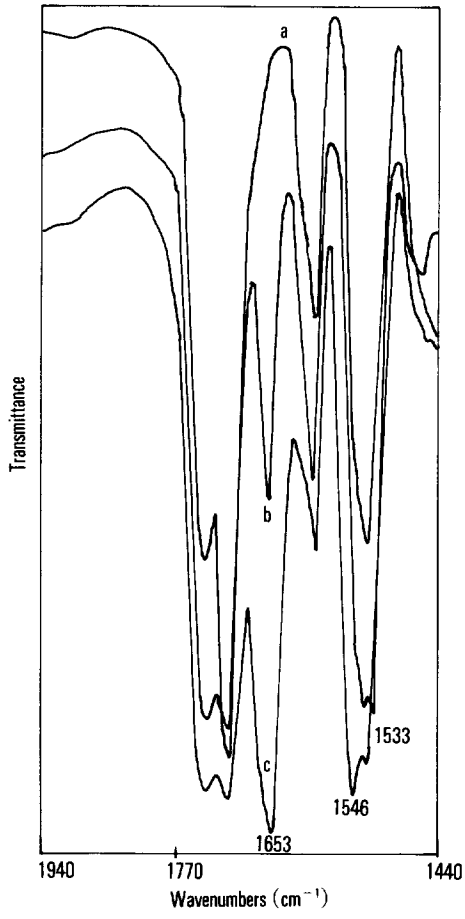

Fig. 5. IR spectra of polyurethane and PGM series ; a) polyurethane, b) PGM-1, and c) PGM-3.

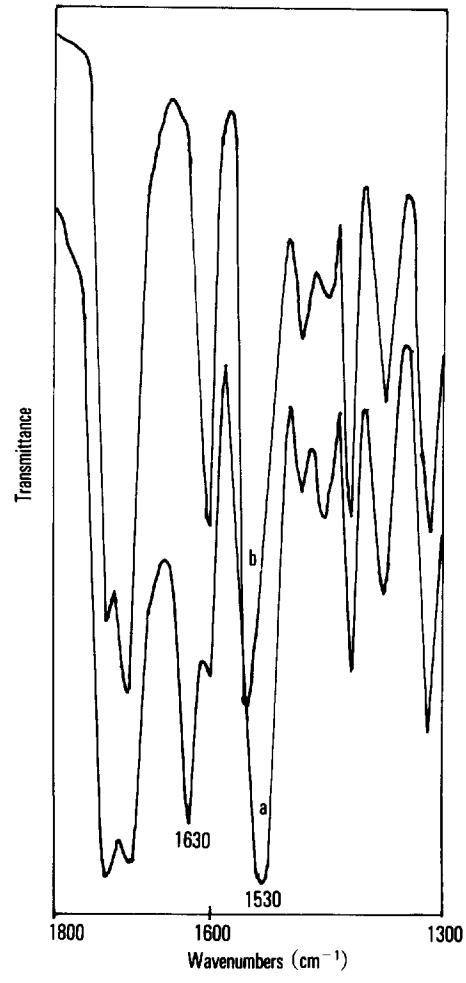

Fig. 6. IR spectra of a) PGE-3 and b) polyurethane.

of the peptide, in general, is relatively low, due to the inter- and intra-molecular hydrogen bonding in the polymer chain.

The degree of water absorption and the critical surface tension of the samples were shown in Table 2. Experimental error of the degree of water absorption and critical surface tension were within seven and five percent, respectively. For all the samples, the degree of water absorption decreased with increasing peptide content in the copolymers. It is interesting to note that the degree of water absorption of PGB series that have an α-helical conformation is always greater than that of PGM and PGE series which possess β-sheet conformation. We speculate that one of the possible reason for this phenomenon is attributed to second order conformational difference of grafted peptide. The critical surface tension of PGB, PGM and PGE series is in the range of 27-28 dyn/cm, 23-26 dyn/cm and 16-18 dyn/cm, respectively. This result is in agreement with the data reported previously.[17,18]

Blood Compatibility

In this study, Lee-White method and microsphere column method were utilized to evaluate the blood compatibility of graft copolymers in vitro. The results are shown in Table 3. The clotting

time of PGB series(ca. 80 min) was much longer than much that of commercial polyurethane(25 min).

Table 2. Degree of Water Absorption and Critical Surface Tension of Samples

Samples	Degree of Water Absorption(%)	Critical Surface Tension(dyn./cm)
PU	3.3	29.5
PGB-1	2.9	28.1
PGB-2	2.4	29.9
PGB-3	2.5	27.4
PGB-4	2.8	27.9
PGB-5	2.5	27.8
PGM-1	2.8	26.1
PGM-2	0.8	26.6
PGM-3	0.1	23.9
PGE-1	0.5	17.2
PGE-2	~0	17.8
PGE-3	~0	17.8

Table 3. The Platelets Adhesion Performance and the Clotting Time Ratio of Samples

Samples	Platelets Adhesion Performance[a] (%)	Clotting Time Ratio[b]
PU	8.8 ± 2	1.00
PGB-1	1.9 ± 1	3.80
PGB-2	4.9 ± 2	3.80
PGB-3	2.4 ± 1	4.08
PGB-4	4.6 ± 1	4.50
PGB-5	9.7 ± 2	2.17
PGM-1	8.2 ± 2	1.42
PGM-2	6.4 ± 2	1.26
PGM-3	3.0 ± 1	1.76
PGE-1	3.0 ± 1	1.10
PGE-2	4.0 ± 1	1.00
PGE-3	4.0 ± 1	1.30

[a] [number of platelets in the column]/[number of platelets in the fresh blood]

[b] [clotting time of polymer samples]/[clotting time of polyurethane] where polyurethane has the clotting time of 25 min.

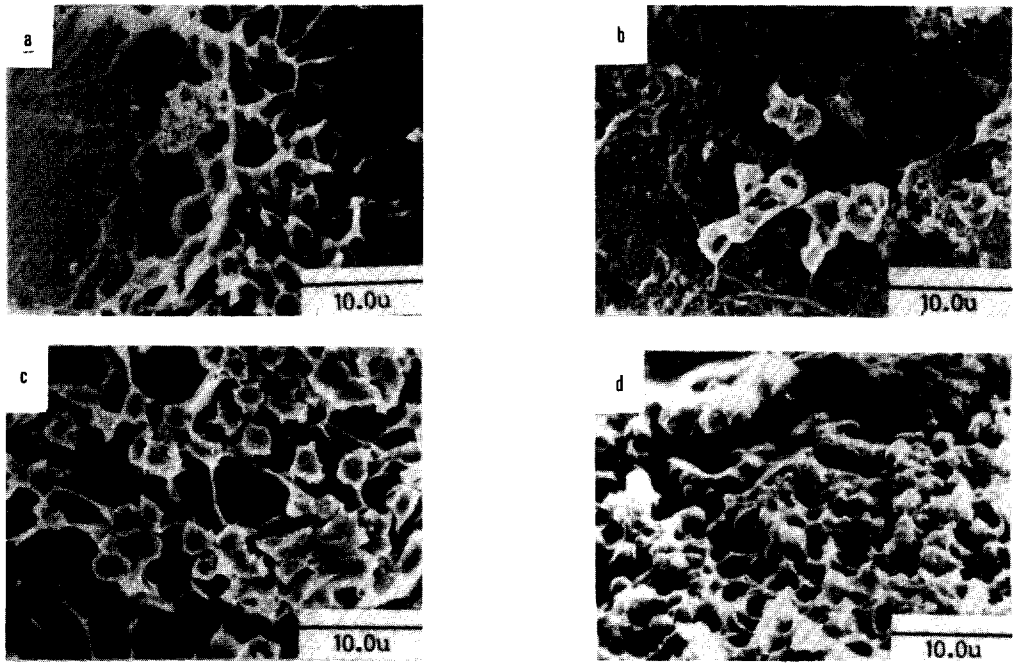

Fig. 7. Scanning electron microscope pictures of platelets adhered onto polyurethane and peptides ; a) polyurethane, b) PBLG, c) PMLG, and d) PELG.

The adhesion of platelets on the polymers is estimated as the percentage of the number of platelets adhered onto the glass beads and glass tube to the number of platelets in the fresh whole blood. The percentage of platelets adhesion on PGB series was less than ten percent. On the other hand, 3-8% of platelets was adhered to PGM and PGE. It is obvious, from the Table 3, that the blood compatibility of the graft copolymer is superior to the unmodified polyurethane.

The platelets adhesion to PGB series measured by column method(a dynamic state) was not significantly different from those of PGM and PGE series. However, the clotting time ratio of PGB series was greater than that of PGM and PGE series. In the static blood compatibility test, the blood compatibility of graft copolymers is expected to be influenced by the conformation of peptide in the graft copolymers.

The morphology of platelets adhered on the surfaces of graft copolymers (PGB, PGM and PGE) was investigated with SEM and shown in Figures 7-9. The fibrin networks are not seen on the surfaces of the graft copolymers although the morphology of platelets is changed to a small extent.

In order to compare the blood compatibility and mechanical properties of commercial polyurethane (Pellethane 2363 80 AE) with the peptide grafted polyurethane, the clotting time ratio of the copolymers was plotted against their tensile strength as shown in Figure 10. The clotting time of the graft copolymer is longer than that of polyurethane, but the mechanical properties are not. However, PGB-1 has a comparable mechanical properties with the commercial polyurethane and longer clotting time than polyurethane.

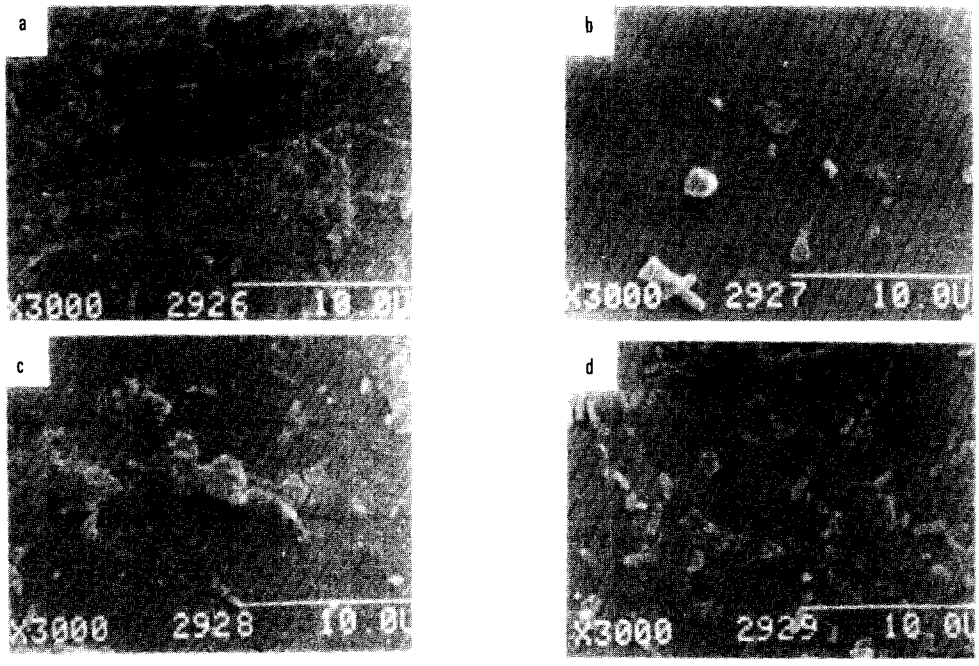

Fig. 8. Scanning electron microscope pictures of platelets adhered onto PGB series ; a) PGB-1, b) PGB-2, c) PGB-3, and d) PGB-5.

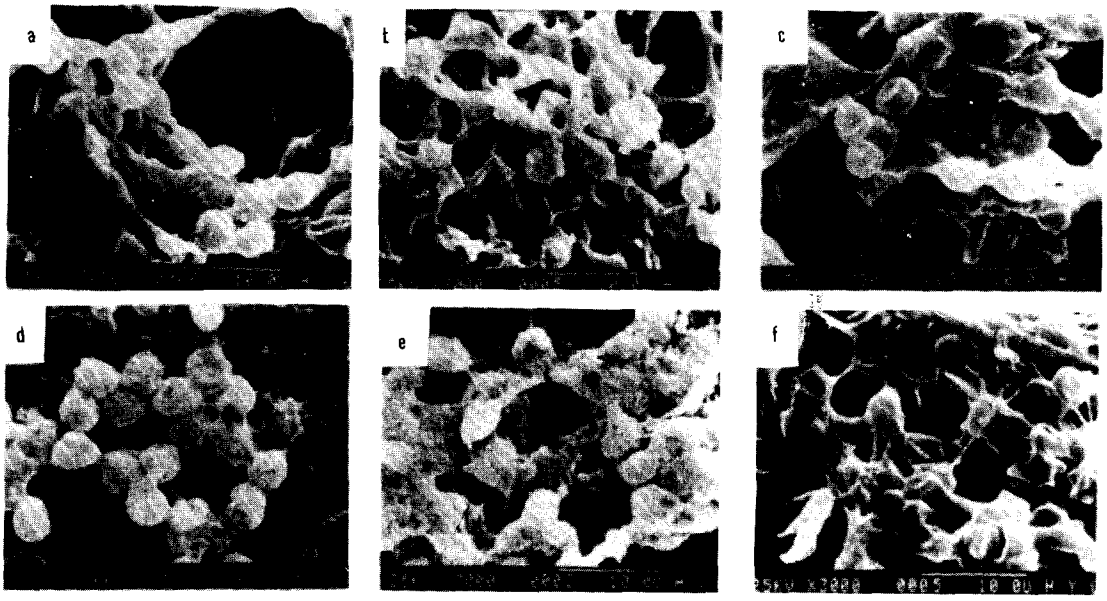

Fig. 9. Scanning electron microscope pictures of platelets adhered onto PGM and PGE series ; a) PGM-1, b) PGM-2, c) PGM-3, d) PGE-1, e) PGE-2 and f) PGE-3.

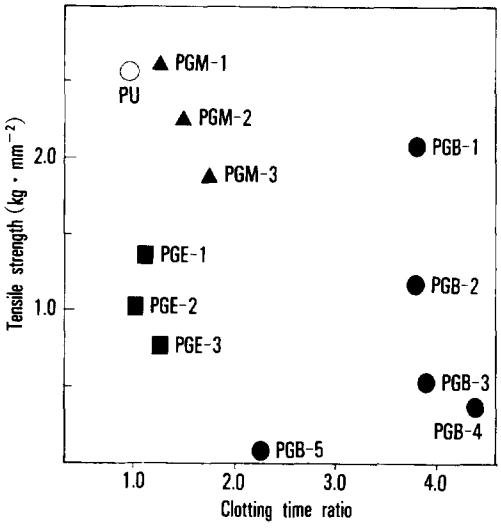

Fig. 10. The relation between clotting time ratio and mechanical properties.

CONCLUSION

Novel graft copolymers consisting of the commercially available polyurethane(Pellethane 2363 80 AE) and γ-alkyl- or γ-benzyl-L-glutamate were synthesized, and their conformations, mechanical properties and blood compatibilities were investigated. The peptide segments of graft copolymers showed an α-helical conformation for PGB series, a β-sheet conformation for PGE series and a combination of an α-helical and random coil conformation for PGM series samples as evidenced by FT-IR spectra. The elongation and tensile strength of the graft copolymers were decreased with increasing the content of the peptide in the copolymers. The degree of water absorption of the samples showed less than three percent. The adhesion of platelets on the PGB series were less than ten percent. In the SEM observation, the fibrin networks and adhered platelets were not found on the surface of PGB series. The clotting time of peptide grafted polyurethanes was longer than that of polyurethane and peptide. Among peptide grafted polyurethane, the PGB series having α-helical conformation exhibited a longest blood clotting time.

REFERENCES

1. Spectrx®-SX System Brochure 81068, Medtronic, Inc., Minneapolis, Minn.
2. E. W. C. Wong, *Am. Chem. Soc. Div. Org. Coat. Plast. Chem. Prepr.*, **43**, 942 (1980).
3. D. K. Gilding, J. A. Taylor, U. S. patent, 3,752,786 (1973).
4. J. W. Boretos, W. S. Pierce, R. E. Baier, A. F. LeRoy, and H. J. Donachy, *J. Biomed Mater. Res.*, **9**, 327 (1975).
5. K. Y. Kim, Y. M. Lee, Y. G. Lee, and K. J. In, *Polymer(Korea)*, **7**, 53 (1983).
6. K. Y. Kim, S. W. Jang, C. S. Cho, S. C. Song, and Y. K. Sung., *Polymer(Korea)*, **13**, 134 (1989).
7. K. Y. Kim, S. W. Jang, D. S. So, C. S. Cho, and Y. K. Sung., *Polymer(Korea)*, **13**, 529 (1989).
8. K. Y. Kim, S. W. Jang, Y. J. Lee, Y. M. Lee, and J. S. Kim, *Polymer J.* (Tokyo), **23**(4), 259 (1991).
9. J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids", p 936, John Wiley and Sons, Inc., 1969.
10. R. B. Woodward and C. H. Scharm, *J. Am. Chem. Soc.*, **69**, 1551 (1947).
11. R. Blumstein, Ph. D. Thesis, University of Delaware, Delaware, U.S.A., June, 1965.
12. W. A. Zisman and H. Y. Fox, *J. Colloid. Sci.*, **7**, 109 (1952).
13. R. I. Lee and D. D. White, *J. Am. Med. Sci.*, **145**, 495 (1913).
14. K. Kataoka, *Makromol. Chem.*, **179**, 1121 (1978).
15. T. Komodo, K. Y. Kim, and T. Kawai, *Makromol. Chem.*, **176**, 373 (1975).
16. A. Asabourian and E. R. Broat, *J. Am. Chem. Soc.*, **78**, 145 (1956).
17. K. Y. Kim, T. Komodo, and T. Kawai, *Makromol. Chem.*, **178**, 2089 (1977).
18. K. Y. Kim and K. J. In, *Polymer(Korea)*, **5**, 459 (1981).